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GENETIC DIVERSITY OF SELECTED FLAX GENOTYPES (*Linu-musitatissimum* L.) BASED ON SCOT, ISSR AND RAMP MARKERS

MARWA H. A. OSMAN[1], M. A. BADAWI[2], E. M. ZAYED[3], REEM M. ABD EL-MAKSOUD[1] AND K.H. RADWAN[4]*

1. Nucleic Acid and Protein ChemistryDepartment, Agricultural Genetic Engineering Research Institute (AGERI), Agricultural Research Center (ARC), Giza, Egypt.

2.Genome mapping Department, Agricultural Genetic Engineering Research Institute (AGERI), Agricultural Research Center (ARC), Giza -12619, Egypt

3.Cell Study Research Department (CSRD), Field Crops Research Institute, Agriculture Research Center (ARC), Giza 12619, Egypt.

4.*Microbial Molecular BiologyDepartment, Agricultural Genetic Engineering Research Institute* (AGERI), Agricultural Research Center (ARC), Giza, Egypt;

** Corresponding author at: Microbial Molecular Biology Department, AGERI, ARC. Giza -12619 Egypt. Tel. 0020235727831, e-mail address:* khradwan@gmail.com

Genus *Linum* belongs to the family *Linaceae* which has 22 genera. It includes three species, *Linum usitatissimum*, the ornamentals *Linum grandiflorum* and *Linum perenne* (El Sayed *et al*., 2018). Flax (*Linum usitatissimum* L.) is a self-pollinated crop with diploid chromosome number 2n=30 (Yadava *et al*., 2012 and Kumari *et al*., 2018). Cultivated flax is an annual crop that has three types; one is grown for oil (linseed), the second for fiber (fiber flax) and the third is grown for both purposes.

Breeding programs require prior knowledge of crop distribution and its genetic diversity (Hoque *et al*., 2020). In general, genetic biodiversity techniques provide information about the useful genes in germplasm resources. Such genes can be transferred during breeding programs through marker-assisted selection by backcrossing (Rahman *et al*., 2016). Traditional methods to assess the genetic variation by using morphological and biochemical markers have the disadvantages of being environmentally dependent, non-reliable and with limited loci numbers (Bekele and Bekele, 2014). Several molecular markers have been developed including Random Amplified Polymorphic DNAs (RAPDs), Restriction Fragment Length Polymorphisms (RFLPs), Amplified Fragment Length Polymorphisms (AFLPs) and Simple Sequence Repeats (SSRs) (Mhiretand Heslop-Harrison, 2018). Start codon targeted (SCoT) marker is one of the reliable techniques as it is considered an efficient, informative and inexpensive tool. The primers used in this method are designed according to the short-conserved region surrounding the ATG translation start codon (Collard and Mackill, 2009; Bhattacharyya *et al*., 2013 and Rathore *et al*., 2014). Inter simple sequence repeat (ISSR) offers several advantages such as high reproducibility, high polymorphism, low DNA requirements, easy handling and high genomic distribution (Heidari*et al*., 2016). However, the random amplified microsatellite polymorphism (RAMP) method combines the advantage of RAPD and ISSR (Ismail *et al*., 2016). Different molecular marker systems vary in their mechanisms of detecting polymorphism and genome coverage in plants. They complement each other to generate accurate specific markers in plants. Many researchers are using several PCR based-markers in the same study to detect polymorphism at the plant genome sequence level. Mao *et al*., (2018) used a combination of ISSR and SCoT markers to offer detailed results than a single analysis of ISSR and SCoT to study genetic diversity and population structure analyses in *Senna obtusifolia* L. Although, many types of molecular markers have been developed and are widely used in plant breeding, most of these marker systems are restricted in their applications because of the limitations on their availability and the high cost of analyses conducted on a large scale (Agarwal *et al*., 2008 and-Sonah*et al*., 2011).

In this study, three DNA molecular markers (SCoT, ISSR and RAMP) were used to evaluate and estimate the genetic diversity among selected flax cultivars currently active in the Egyptian flax breeding programs.

MATERIAL AND METHODS

Plant materials

Twelve flax (*Linum usitatissimum*L.) genotypes were obtained from the germplasm collection of the Field Crops Research Institute (FCRI), Agricultural Research Center (ARC), Egypt. These genotypes represent the most promising flax genotypes and were originally introduced from the Netherlands to be integrat-

ed into the flax breeding programs in Egypt. The code, accession number, accession name, country of origin, sample state and sub crops of the selected flax genotypes are presented in Table (1).

DNA extraction

Genomic DNA was isolated from freshly harvested leaves of seven-day-old seedlings. The leaves were ground to a fine powder and the DNA was isolated using QIAGEN DNeasy plant Mini Kit. The concentration and purity of the genomic DNA samples were estimated by the Thermo Scientific™ NanoDrop 2000 spectrophotometer.

SCoT, ISSR and RAMP-PCR assays

Thirteen SCoT, ISSR primers and eleven RAMP primer combinations were used to screen the twelve flax genotypes (Tables 2, 3 and 4, respectively). PCR amplification reactions were conducted using 1X PCR reaction buffer, 1.5 mM $MgCl_2$, 200 µM dNTPs mix, 2 µM SCoT and ISSR primers while 1µM for each primers combination of RAMP was used, 50ng template DNA, 1.5 units GoTaq® DNA polymerase in a total reaction volume of 25 µl.

PCR conditions

PCR amplifications were carried out in Gene Amp® PCR System 9700 thermal cycler (Applied Biosystems). The PCR program used for SCoT was as follows: initial denaturation at 94ºC for 5min followed by 40 cycles of denaturation at 94ºC for 40 sec., annealing at 50ºC for 50 sec., extension at 72ºC for 1 min and a final elongation step at 72ºC for 7 min followed by a final soak at 12ºC.

The PCR program for ISSR amplification was as follows: one denaturation cycle at 94ºC for 5 min, followed by 40 cycles at 94ºC for 45 sec., annealing at 38ºC for 1 min and extension cycle at 72ºC for 2 min. The final extension cycle was carried out at 72ºC for 7 min.

PCR RAMP amplification was programmed for 40 cycles after an initial denaturation cycle of 5 min at 94ºC. Each cycle consisted of denaturation at 94ºC for 1 min, annealing at 38ºC for 1 min and a final extension at 72ºC for 2 min, followed by a final elongation cycle at 72ºC for 7 min.

The PCR products of the three different molecular markers were analyzed on 1.5% agarose gel and the molecular weights of fragments were estimated with GeneRuler 1kb DNA ladder (Thermo Scientific). Gels were stained with ethidium bromide and photographed using Gel Documentation System (BIO-RAD Gel Doc XR^+).

Data analysis

Amplified products for SCoT, ISSR and RAMP markers were visually scored based on the presence or absence of bands. Genetic parameters; Ne and Na (Hartl and Clark, 1997) and Shannon index I (Shannon, 1949) were calculated by the POPGENE software (version 1.32)

(Yeh *et al*., 1997) as well as the PIC version 3.25 (Liu and Muse, 2005) according to Botstein *et al*., (1980). The average number of alleles per locus, allele frequency (pi), expected heterozygosity (H), the effective number of alleles per locus (Ne) were also calculated as described by Morgante*et al*., (1994). Moreover, the multiplex ratio (MR) and the effective multiplex ratio (EMR) were calculated according to Powell *et al*.,(1996). The marker index (MI) was used to calculate the overall utility of a marker system depending on the formula; MI= EMR x PIC (Powell *et al*., 1996). The relationships between the distance matrixes were analyzed by the Power Marker program according to Mantel, (1967). Pairwise comparison of different genotypes was performed based on the absence (0) or the presence (1) or of unique and shared polymorphic products to construct a similarity coefficient using statistical software package STATISTICA- SPSS (Stat Soft Inc.). The generated similarity coefficient was used to construct a dendrogram by the Un-weighted Pair Group Method with arithmetic averages (UPGMA) according to Nei and Li, (1979).

RESULTS AND DISCUSSION

DNA polymorphism was used to assess the pattern of genetic diversity obscured by the complexities of pedigree records using the three PCR-based markers for the main 12 elite flax genotypes actively used in the breeding programs in Egypt.

Genetic polymorphism

The thirteen primers for each of SCoT (Fig. 1 and Table 2) and ISSR (Fig. 2 and Table 3) markers, and the eleven RAMP primer combinations (Fig. 3 and Table 4), produced a total number of 209, 177 and 143 bands, respectively. The highest number of polymorphic bands was recorded by SCoT (168) with a polymorphic percentage of 80.4%. While the ISSR marker recorded 114 polymorphic bands representing a polymorphic percentage of 64.4%. On the other hand, the lowest number of polymorphic bands was that produced by RAMP (87) representing a total polymorphism of 60.8%. The lowest percentage of polymorphism recorded by SCoT (50%) was revealed by primer SCoT 16. Interestingly, primer SCoT 22 showed a high polymorphism of 100%. However, the highest percentage of polymorphism recorded by the ISSR primer ISSR10 (86.4%), while the lowest (50%) was recorded by primer ISSR 11. On the other hand, primer combinations RAMP 02 showed the highest percentage of polymorphism (87.5%), while primer combination RAMP 09 recorded the lowest percentage of polymorphism (50%). The average number of polymorphic bands produced per primer was 12.9 (SCoT), 8.8 (ISSR) and 7.9 (RAMP). In conclusion, the used primers revealed a convergent level of polymorphism among the studied 12 flax genotypes.

These data were similar to that obtained by Ahmed *et al*., (2019) analyzing

the genetic diversity within nine flax genotypes. They used 10 SCoT primers and detected a total of 120 DNA fragments with an average of 12 bands per primer compared to 209 bands that were derived through this work. Moreover, the SCoT marker was used to examine the genetic variation amongst different crops; Mohamed *et al*., (2017) used thirteen SCoT primers to examine the genetic polymorphism among nine olive cultivars and successfully reported a total of 242 bands with an average of 18.6 bands per primer with a high average polymorphism percentage (97.52%). Also, Agarwal, (2019) and Zhao *et al*., (2020) reported higher polymorphism percentages (92.20% and 100%, respectively) revealing the elevated level of variability within similar studies using the SCoT marker which could be attributed to the differences in genotypes studied.

These findings are also in agreement with that of Rajwade*et al*., (2010) who reported a polymorphism percentage of 63.9% working on 70 Indian flax accessions using 12 ISSR primers.Wiesnerová and Wiesner, (2004) and Pali and Mehta, (2016) reported higher percentages of polymorphism (72.6 and 90%, respectively). Kumari *et al*., (2018) used 11 ISSR primers and detected 58 fragments with an average number of 5.2 per primer studying 28 flax genotypes. Also, Ahmed *et al*., (2019) recorded 54.8% ISSR polymorphism among the nine flax genotypes they studied.

Moreover, Mohamed *et al*., (2017) used eleven RAMP primer combinations to study polymorphism among olive cultivars and obtained a polymorphism percentage of 81.39%. In this context, Rhouma-Chatti*et al*., (2011) obtained 210 reproducible bands using 18 RAMP primer combinations to study genetic diversity within Tunisian date-palm cultivars and reported a polymorphism percentage of 88.57. In addition, Rai *et al*., (2013) used 17 RAMP primer combinations and obtained 106 fragments; of which 87 were polymorphic while studying 48 Capsicum genotypes. Also, Saleh, (2015) used 21 RAMP primer combinations and successfully distinguished 145 loci, with a polymorphic percentage of 95.9 in a study on *Arthrocnemummacrostachyum*genotypes. Liu *et al*., (2020), studying 16 *Lycium*species, obtained a polymorphic percentage of 89.05 using RAMP primer combinations.

SCoT, ISSR and RAMP markers utilized in the present study can be used to characterize and quantify genetic diversity in flax germplasm of any origin and region. Moreover, the high polymorphism observed highlights the allelic richness of the analyzed germplasm, which is a useful aspect for this crop improvement program.

Cultivar identification

The three different types of molecular markers; SCoT, ISSR and RAMP were applied in the present study to develop unique molecular markers to be further used in genotype identification as well as

to generate a unique fingerprint for each flax genotype.

Results presented in Table (2) show that eight flax genotypes (G1, G4, G6, G7, G8, G9, G10 and G12) were successfully characterized by a total of 28 unique SCoT markers including 8 positives and 20 negative markers. Unique markers; ranged in size from 150 to 2000 bp; successfully characterized 8 out of the 12 genotypes. Primer SCoT11 identified four genotypes, while primers SCoT 3, 12, 22, 28 successfully characterized three genotypes each. Also, both primers SCoT 4 and 20 identified genotypes G7 and G12; primers SCoT 2, 5 and 35 identified one genotype each. Genotype G7 was identified by the highest number of negative unique markers (9) in addition to two positive markers, followed by genotype G12 which was identified by four negative markers in addition to three positive markers. Genotypes G1, G6 and G9 were characterized by only one positive marker while genotype 8 was characterized by only one negative marker. Genotype G4 was characterized by four negative markers and finally, genotype G10 was characterized by two negative markers.

Among the 13 applied ISSR primers (Table 3), only three revealed unique markers characterizing 4 out of the 12 flax genotypes (G3, G7, G9 and G11). These were identified by a total of 3 positive markers and one negative marker. These markers ranged in size from 700 to 2000 bp. Genotypes G7, G9 and G11 were characterized by only one positive marker. Moreover, genotype G3 was distinguished by only one negative marker.

As shown in Table (4), RAMP markers permitted the distinction of 5 genotypes (G1, G5, G6, G9 and G12) by twelve specific unique markers. These markers ranged in size from 250 to 2500 bp. Genotype G7 was characterized by the highest number of both positive and negative unique markers (3). Genotype G9 showed one positive marker and one negative marker. Genotype G12 showed 2 positive markers, while genotypes G1 and G6 were characterized by only one positive marker each.

The three molecular markers under study successfully generated specific unique fingerprints that can be used to identify the 12 flax genotypes and could be useful in plant breeding programs. These results are in accordance with those of Rai *et al*., (2013), Saleh, (2015), Mohamed *et al*., (2017), Agarwal*et al*., (2019) and Liu *et al*., (2020).

Genetic relationships among flax genotypes

While the genetic similarity revealed by SCoT marker ranged from 0.608 (between G11 and G7) to 0.836 (between G1 and G3), the genetic similarity assessed by ISSR marker for the 12 flax genotypes ranged from 0.705 to 0.903. The lowest genetic similarity (0.705) appeared between G1 and G7 while the highest (0.903) was between G8 and G9. On the other hand, the genetic similarity

of RAMP markers ranged from 0.70 between G2 and G12 to 0.95 between G5 and G10.

In this context, Mohamed *et al*., (2017) obtained genetic similarities based on SCoT, ISSR and RAMP that scored an average of 0.62, 0.81 and 0.78, respectively. Rajawade*et al*., (2010) obtained a genetic similarity matrix that ranged from 0.60 to 0.97 among 70 Indian flax accessions using 12 ISSR primers. Pali and Mehta (2016) obtained a similarity ranged from 0.56 to 0.97 with an average of 0.76 studying 48 flax genotypes based on the ISSR marker. Rhouma-Chatti*et al*., (2011) reported that genetic similarity ranged from 0.10 to 0.76 with a mean of 0.34 for date-palm genotypes using RAMP marker.

The dendrogram of genetic relationships derived from SCoT data revealed two main clusters, one of them isolated only the genotype G7, representing an oilseed flax landrace originated from the Netherlands, while the other cluster contained the remaining 11 flax genotypes (Fig. 4A). The latter was further divided into two sub-clusters; the first one separated only one genotype (G12) representing a breeder line for fiber flax originating from Canada and the second one contained two groups, one comprised of three genotypes G2, G6 and G10 combining fiber flax originated from the Netherlands and Northern Ireland, while the other group included the remaining genotypes.

The same findings were obtained by El-framawy*et al*., (2016) who constructed a dendrogram based on the UPGMA analysis of the SCoT results studying *Atriplex halimus* populations that were divided into two main genetic groups. Moreover, Agarwal *et al*., (2019) successfully constructed a dendrogram representing the relationship amongst 29 rose genotypes based on SCoT marker data. They also reported a dendrogram that grouped the studied cultivars into two main clusters.

The derived ISSRs dendrogram (Fig. 4B) divided the 12 flax genotypes into two main clusters, one cluster containing the two genotypes; G7 and G12, originating from the Netherlands and Canada, and the second was further divided into two sub-clusters; one contained three genotypes G2, G6 and G10, while the other was further divided into two more groups, one separating the fiber breeder flax line originating from Argentina (G4) and the other contained genotypes G1, G3, G5, G8, G9, and G11. Pali and Mehta, (2016) clustered 48 flax genotypes into two major groups based on the similarity index data derived from SSR and ISSR markers. Also, Ahmed *et al*., (2019) obtained a two-grouped dendrogram amongst nine flax genotypes based on ISSR marker data.

Similarly, The dendrogram derived from RAMP marker data separated the 12 flax genotypes into two main clusters (Fig. 4C), the first one included genotypes G7 and G12, while the second was further

divided into two subclusters, one contained genotype G2 and G3 representing the fiber flax breeder line originating from the Netherlands and the other subcluster was further divided into two groups, one group contained the genotypes G8, G9, G10 and G11 representing fiber flax originating from India, Northern Ireland and Germany while the other group contained genotypes G1, G4, G5 and G6 originating from Egypt, Argentina, Germany and Netherlands. Pu *et al*., (2009), Rhouma-Chatti*et al*., (2011) and Ismail *et al*., (2016) used data derived from RAMP to construct a dendrogram that was separated into two major groups working on 46 barley genotypes, Tunisian date-palm germplasm and eight populations of *Clinacanthus nutans*, respectively.

Even though the topology of the four dendrograms was different and that each molecular marker represented a unique pattern, dendrograms shared the same position for genotypes G7 and G12 which were always separated together with a lower genetic distance. Although G7 is an oilseed flax landrace from the Netherlands whereas, G12 is a fiber flax breeder line originating from Canada.

Combined dendrograms further confirmed that genotypes G7 and G12 are separated from the other studied genotypes studied. Also, it clustered genotypes G2, G6 and G 10 in a separate group. Interestingly, it revealed that the closest genotype to Giza (G1) was G3 (Netherlands). It is worth mentioning that genotype G1 (Giza) originating from Egypt behaved differently with each technique used indicating that it has a common genetic background with the different genotypes studied. These discrepancies in the genetic similarity revealed by the different marker types could be attributed to the different mechanisms of detecting the polymorphism and genome coverage offered by each marker. Therefore, the genetic similarity based on the combined data could be more representative of the genetic relationships.

Comparison

Combining the results of the three different PCR-based markers (ISSR, SCoT and RAMP) allowed a better comparison than that of each genetic marker alone in characterizing flax cultivars. These markers successfully provided a unique fingerprint for each flax genotype studied. The comparative analysis presented in Table (5) summarizes the resultsof the comined data of the 13 SCoT, 13 ISSR primers and 11 RAMP primer combinations showing that they successfully produced a total number of 209, 177 and 143 reproducible bands, with a percentage of polymorphism of 80.38%, 64.40% and 60.83%, respectively. The high polymorphism generated by these markers indicates that these flax cultivars are polymorphic. The discriminatory power of each marker was evaluated by comparing its PIC mean and MI values. The highest value of polymorphism was that recorded by SCoT marker with a PIC value of (0.23) and MI of (2.96) compared to ISSR (0.20 and 1.74) and RAMP (0.16

and 1.06) which indicated that these loci were more informative for the flax cultivars studied. Thus, the SCoT marker can be used as an effective complementary method besides ISSR and RAMP for molecular characterization of flax as well as for the determination of genetic relationships between their cultivars.

Also, the data derived from this work showed higher SCoT values of Na (1.82), Ne (1.51) and EMR (12.9) compared to that of ISSR (1.65, 1.41 and 8.7, respectively) and RAMP (1.63, 1.34 and 6, respectively). The Shannon index produced by ISSR (0.35) showed higher variability than that produced by SCoT (0.25) and RAMP (0.30).

These findings correlate with the data reported by Gorji *et al*., (2011)stating that the SCoT marker was the most informative marker based on the average percentage of polymorphism, PIC and Shannon index data derived from studying potato populations. Also, Alikhani*et al*., (2014) reported that SCoT markers were more informative than ISSR markers for diversity assessment among Persian oak (*Quercus brantii*L.) individuals where it recorded the highest PIC value (0.38) compared to ISSR (0.30). On the contrary, Ismail *et al*., (2016) reported a high value of PIC of 0.25, MI of 11.36 using data derived from RAMP marker compared to that derived from RAPD and ISSR studying *clinecanthus nutans* populations. Consequently, these results agree with that previously reported by Guo *et al*., (2012), Ismail *et al*., (2016) and Cheginі*et al*., (2016) in which PIC, EMR, and MI were anticipated as the most accountable marker parameters for selecting informative markers.

Also, conducting genetic diversity analyses on flax genotypes; Ahmed *et al*., (2018)reported higher Ne, PIC and MI values of 206.1, 0.948 and 6.60,respectively using the SCoT marker. Nevertheless, Ahmed *et al*., (2019) by using the ISSR markers obtained values of 142.6, 0.89 and 3.96 for Ne, PIC and MI values, respectively. Singh *et al*., (2017) recorded close values of polymorphism among SCoT and ISSR markers (61.6% and 61%, respectively). However, they reported a higher PIC value of 90.4% for SCoT compared to 88.8% for the ISSR marker. On the other hand, Agarwal*et al*., (2019) reported that ISSR markers to be more informative in assessing genetic diversity among 52*Trichosanthesdioica*Roxb accessions whereas they produced a higher PIC value (0.47) compared to that of SCoT markers (0.45).

In this study, the SCoT marker proved to be more accountable than ISSR and RAMP markers in studying genetic polymorphism among flax cultivars. This may be due to the nature of the SCoT marker that being a gene-targeted one. These results are considered valuable regarding establishing a molecular database for the identification of the most promis-

ing flax cultivars used in breeding programs in Egypt.

As conclusion**,** Genetic characterization is the key to addressing concerns about flax breeding programs. Flax genotypes characterized and analyzed in the present study showed a broad genetic diversity spectrum. Eight flax genotypes were successfully characterized by SCoT markers; four genotypes were characterized by ISSR primers while RAMP markers permitted the distinction of five flax genotypes. SCoT, ISSR and RAMP markers are considered to be powerful tools for the discrimination and identification of the studiedflax genotypes. However, the SCoT marker is the recommended in assessing the genetic diversity of flax genotypes over ISSR and RAMP. The obtained markers will be useful for devising conservation strategies for the genetic improvement of flax in Egypt.

SUMMARY

Thirteen Start Codon Targeted (SCoT), thirteen inter simple sequence repeat (ISSR) primers and eleven random amplified microsatellite polymorphism (RAMP) primer combinations were used to assess the genetic diversity among twelve Flax (*Linum usitatissimum* L.) genotypes that are currently active in flax breeding programs in Egypt. They successfully produced a total of 209, 177 and 143 reproducible bands, with a polymorphism percentage of 80.38%, 64.40% and 60.83%, respectively. The highest polymorphism was obtained by SCoT marker with a polymorphic information content (PIC) value of (0.23) and marker index (MI) of (2.96) compared with ISSR markers (0.20 and 1.74, respectively) and RAMP (0.16 and 1.06, respectively). Also, data showed higher SCoT values of observed alleles (Na) 1.82, the number of effective alleles (Ne) 1.51 and the effective multiplex ratio (EMR) 12.9 compared with ISSRs (1.65, 1.41 and 8.7) and RAMP (1.63, 1.34 and 6.00, respectively). On the other hand, Shanon index (I) produced by ISSR marker (0.35) showed higher variability than SCoT (0.25) and RAMP (0.30). However, dendrograms derived from SCoT, ISSR and RAMP marker data separated the 12 flax genotypes into two main clusters. Thus, SCoT marker can be used as an effective complementary method besides ISSR and RAMP for molecular characterization of flax as well as for the determination of genetic relationships between flax cultivars.

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Table (1):Code, accession number, accession name, country of origin, sample state and sub crop of the selected twelve linum genotypes.

Code	Acc. Num.	Acc. Name	Country of Origin	Sample State	Sub Crop
G1	CGN21272	Giza	Egypt	Advanced cultivar	Oilseed flax
G2	CGN20366	536 Hi	Netherlands	Breeder line	Fibre flax
G3	CGN20368	543 Hi	Netherlands	Breeder line	Fibre flax
G4	CGN19454	H.I. 31-1-3-3-5-1	Argentina	Breeder line	Fibre flax
G5	CGN21141	Hohenheimer 53	Deutsch Land	Advanced cultivar	Fibre flax
G6	CGN21143	Resistenta	Netherlands	Advanced cultivar	Fibre flax
G7	CGN21182	L. cory	Netherlands	Land race	Oilseed flax
G8	CGN19469	L.U. from Bombay	Netherlands	Land race	Fibre flax
G9	CGN20315	Liral Suffolk	GBR, Northern, Ireland	Advanced cultivar	Fibre flax
G10	CGN19370	Lyngby 7	GBR, Northern, Ireland	Breeder line	Fibre flax
G11	CGN20309	Katzenellenbogen	Germany	Land race	Fibre flax
G12	CGN19496	Ottawa 804D	Canada	Breeder line	Fibre flax

Table (2): List of the selected thirteen SCoT primer names, sequence, Total and polymorphic number of amplicons, positive and negative unique markers generated for the twelve selected flax (*Linum usitatissimum* L.) genotypes.

primer	Primer sequence (5'-3')	Total # of amplicons	Polymorphic amplicons	% of polymorphism	Pum (bp)	genotype	Num (bp)	genotype
SCoT 2	CAACAATGGCTACCACCC	13	10	76.9	2000	7	-	-
SCoT 3	CAACAATGGCTACCACCG	16	14	87.5	1100	7	500,650, 750	12,10,7
SCoT 4	CAACAATGGCTACCACCT	23	17	73.9	1500	12	250, 450, 650, 1000	7
SCoT 5	CAACAATGGCTACCACGA	17	14	82.4	-	-	900, 1300	12
SCoT 11	AAGCAATGGCTACCACCA	17	14	82.4	800, 1000	12	350, 600, 1200	4,10,7
SCoT 12	ACGACATGGCGACCAACG	22	19	86.4	150	1	300,900	4,8
SCoT 16	ACCATGGCTACCACCGAC	8	4	50	-		-	-
SCoT 20	ACCATGGCTACCACCGCG	22	19	86.4	-	-	600	7,12
SCoT 22	AACCATGGCTACCACCAC	14	14	100	1100, 1700	6, 9	1000	7
SCoT 28	CCATGGCTACCACCGCCA	8	6	75	-		600, 1000, 1400	4,12,7
SCoT 33	CCATGGCTACCACCGCAG	17	13	76.5	-	-	-	-
SCoT 35	CATGGCTACCACCGGCCC	16	12	75	-	-	1250	4
SCoT 36	GCAACAATGGCTACCAC	16	12	75	-		-	-
Total		209	168	80.4	8	5	20	5

Table (3): List of the selected thirteen ISSR primer names, sequence, total and polymorphic number of amplicons, positive and negative unique markers generated for the selected twelve flax (*Linum usitatissimum* L.) genotypes.

Primer	Primer sequence (5'-3')	Total # of amplicons	Polymorphic amplicons	% of polymorphism	Pum (bp)	genotype	Num (bp)	genotype
ISSR 01	5'-AGAGAGAGAGAGAGAGYC-3'	12	9	75	1100	11	2000	3
ISSR 03	5'-ACACACACACACACACYT-3'	15	8	53.3	-	-	-	-
ISSR 04	5'-ACACACACACACACACYG-3'	10	6	60	-	-	-	-
ISSR 05	5'-GTGTGTGTGTGTGTGTYG-3'	15	10	66.7	700	7		
ISSR 07	5'-GACGATAGATAGATAGATA-3'	8	5	62.5	-	-	-	-
ISSR 08	5'-GACAGACAGACAGACAAT-3'	12	10	83.3	900	9	-	-
ISSR 10	5'-GACAGACAGACAGACAAT-3'	22	19	86.4	-	-	-	-
ISSR 11	5'-ACACACACACACACACYA-3'	12	6	50	-	-	-	-
ISSR 12	5'-ACACACACACACACACYC-3'	16	9	56	-	-	-	-
ISSR 13	5'-AGAGAGAGAGAGAGAGYT-3'	12	7	58	-	-	-	-
ISSR 14	5'-CTCCTCCTCCTCCTCTT-3'	14	9	64	-	-	-	-
ISSR 19	5'-HVHTCCTCCTCCTCCTCC-3'	15	10	66	-	-	-	-
ISSR 20	5'-HVHTGTGTGTGTGTGT-3'	14	6	42	-	-	-	-
Total		177	114	64.40	3	3	1	1

Table (4): List of the selected eleven RAMP primer combination names, sequence, Total and polymorphic number of amplicons, positive and negative unique markers generated for the selected twelve flax (*Linum usitatissimum* L.) genotypes.

Combi-nation number	Primer combina-tion	Primer sequence (5'-3')	Total # of amplicons	Poly-morphic ampli-cons	% of poly-morph ism	Pum (bp)	geno-type	Num (bp)	geno-type
1	OPA14/ ISSR 08	TCTGTGCTGG/ GACAGACAGACAGACAAT	12	9	75.0	1800	7	700	7
2	OPA17/ISSR 14	GACCGCTTGT/ CTCCTCCTCCTCCTCTT	16	14	87.5				
3	OPA18/ISSR 10	AGGTGACCGT/ ACACACACACACACACYG	11	8	72.7				
4	OPB16/ISSR10	TTTGCCCGGA/ ACACACACACACACACYG	11	6	54.5				
5	OPA16/ISSR 10	AGCCAGCGAA/ ACACACACACACACACYG	12	9	75.0	250, 2500	6,7		
6	OPB14/ ISSR 10	TCCGCTCTGG/ ACACACACACACACACYG	10	4	40.0				
7	OPA20/ ISSR 14	GTTGCGATCC/ CTCCTCCTCCTCCTCTT	14	11	78.6				
8	OPB1/ ISSR 14	GTTTCGCTCC/ CTCCTCCTCCTCCTCTT	16	12	75.0	2300, 600	7,12	1600, 2100,	7
9	OPB5/ISSR 08	TGCGCCCTTC/ GACAGACAGACAGACAAT	10	5	50.0	1400	12		
10	OPB7/ISSR 20	GGTGACGCAG/ HVHTGTGTGTGTGTGTGT	16	6	37.5	280	9	300	9
11	OPB12/ ISSR 20	CCTTGACGCA/ HVHTGTGTGTGTGTGTGT	15	3	20.0	1100	1		
Total			143	87	60.8	8	5	4	2

Table (5): Comparison the efficiency of SCoT, ISSR and RAMP to characterize the twelve linum genotypes based on polymorphism parameter used in this investigation.

Parameter	Marker name		
	SCoT	ISSR	RAMP
Number of assays screened	13	13	11
Total loci screened	209	177	143
Multiplex ratio	16.07	13.6	11
Total number of polymorphic loci	168	114	87
Polymorphism per assay (%)	80.38%	64.40%	60.83%
No. of observed alleles (Na)	1.82	1.65	1.63
No. of effective alleles (Ne)	1.51	1.41	1.34
Shannon index (I)	0.25	0.35	0.30
Effective multiplex ratio (EMR)	12.9	8.7	6.6
Polymorphic information content (PIC) mean	0.23	0.20	0.16
Marker index	2.96	1.74	1.06

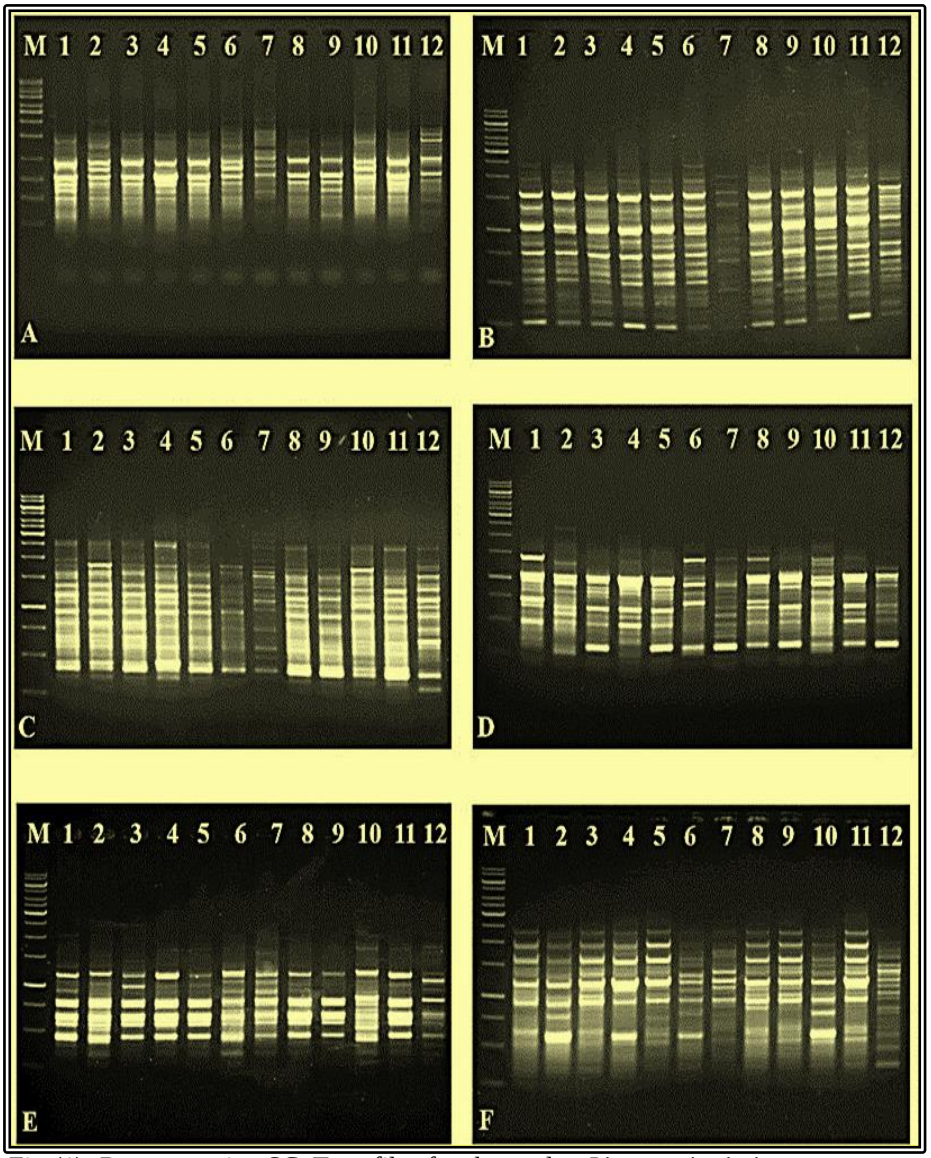

Fig (1): Representative SCoT profiles for the twelve *Linum usitatissimum* genotypes with primers SCoT 03 (A), SCoT 02 (B), SCoT 11 (C), SCoT 12 (D), SCoT 20 (E) and SCoT 33 (F), respectively, M is 1Kb DNA marker. Genotypes are numbered according to Table (1).

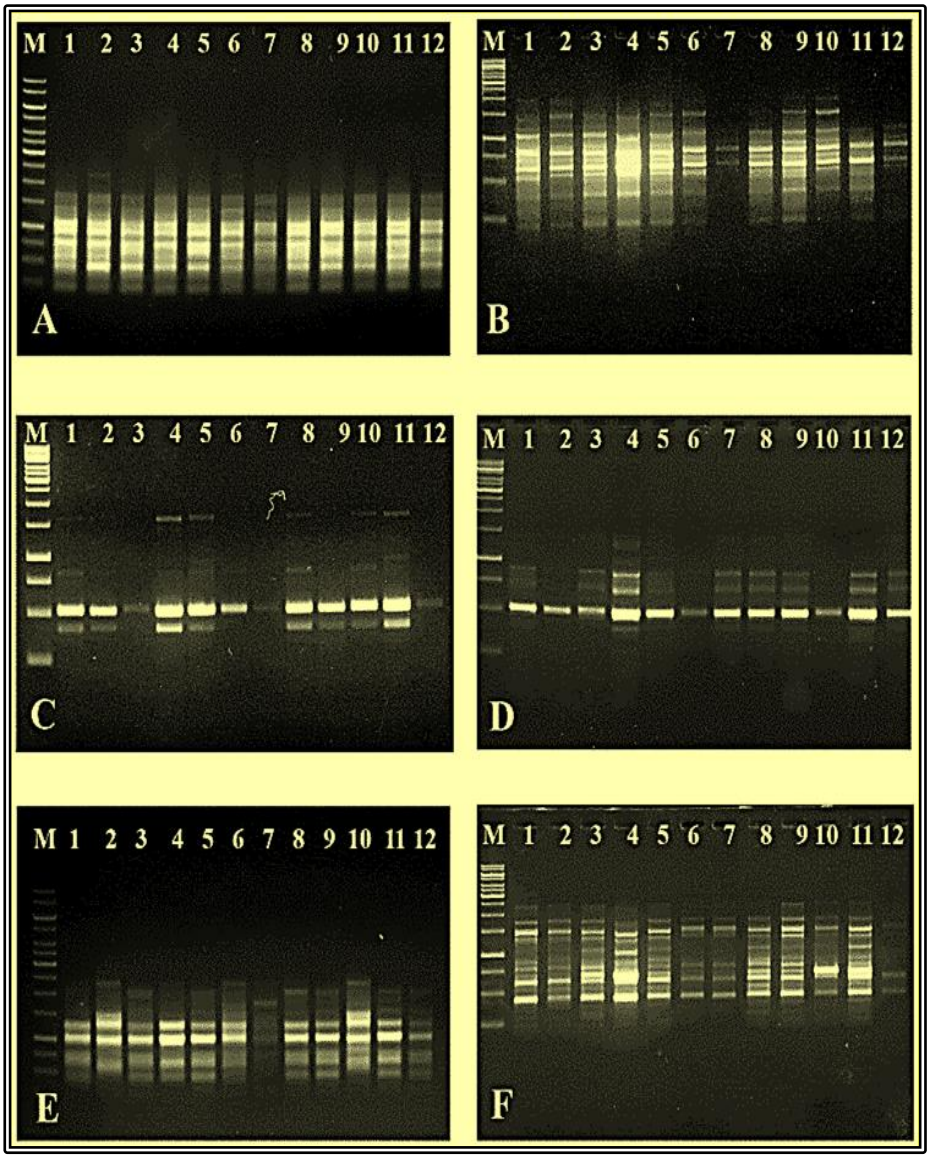

Fig (2): Representative ISSR profiles for the twelve *Linum usitatissimum* genotypes with primers ISSR 01 (A), ISSR 04 (B), ISSR 06 (C) and ISSR 07 (D) ISSR 08 (E) ISSR 14 (F), respectively, M is 1Kb DNA marker. Genotypes are numbered according to Table (1).

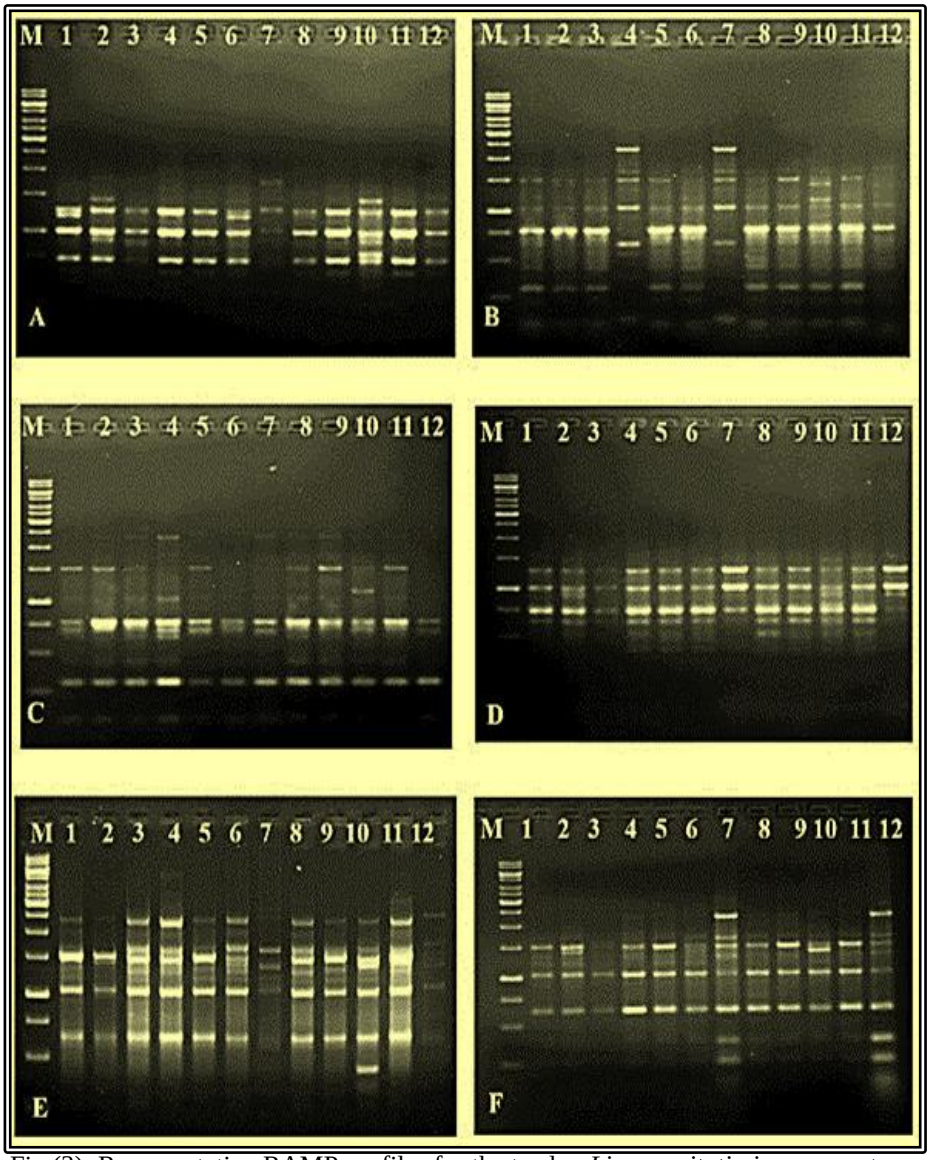

Fig (3): Representative RAMP profiles for the twelve *Linum usitatissimum* genotypes with primer combinations OPA14/ ISSR 08 (A), OPB16/ISSR10 (B), OPA18/ISSR 10 (C) and OPB5/ISSR 08 (D) OPB14/ ISSR 10 (E) and OPB16/ISSR10 (F), respectively, M is 1Kb DNA marker. Genotypes are numbered according to Table (1).

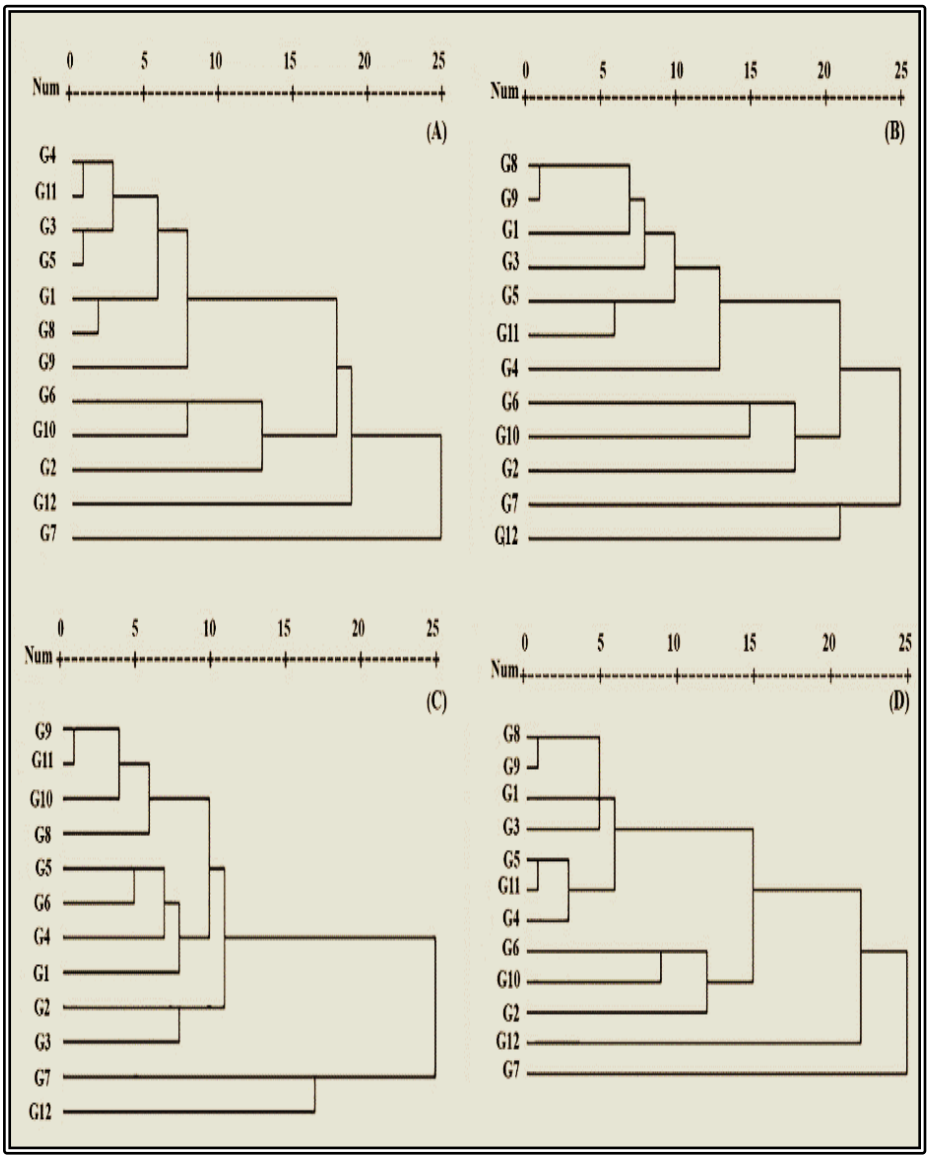

Fig. (4): Dendrogram constructed according to Nei and Li's coefficient using Unweighed Pair-group Arithmetic Average (UPGMA) and the similarity matrix of the SCoT (A), ISSR (B), RAMP (C) and the combined (D) generated by the data of the twelve *Linum usitatissimum* L. genotypes.